(54) Title: SELF-CONTAINING LACTOCOCCUS STRAIN

(57) Abstract: The invention relates to a recombinant *Lactococcus* strain, with environmentally limited growth and viability. More particularly, it relates to a recombinant *Lactococcus* that can only survive in a medium, where well-defined medium compounds are present. A preferred embodiment is a *Lactococcus* that may only survive in a host organism, where said medium compounds are present, but cannot survive outside the host organism in absence of said medium compounds.

SELF-CONTAINING *Lactococcus* STRAIN

Field of the invention

The invention relates to a recombinant *Lactococcus* strain, with environmentally limited

5   growth and viability. More particularly, it relates to a recombinant *Lactococcus* that can only survive in a medium, where well-defined medium compounds are present. A preferred embodiment is a *Lactococcus* that may only survive in a host organism, where said medium compounds are present, but cannot survive outside the host organism in absence of said medium compounds. Moreover, said *Lactococcus*

10   can be transformed with prophylactic and/or therapeutic molecules and can, as such, be used to treat diseases such as inflammatory bowel diseases.

Background of the invention

Lactic acid bacteria have long time been used in a wide variety of industrial

15   fermentation processes. They have generally-regarded-as-safe status, making them potentially useful organisms for the production of commercially important proteins. Indeed, several heterologous proteins, such as Interleukin-2, have been successfully produced in *Lactococcus* spp (Steidler *et al.*, 1995). It is, however, unwanted that such genetically modified micro organisms are surviving and spreading in the environment.

20   To avoid unintentional release of genetically modified microorganisms, special guidelines for safe handling and technical requirements for physical containment are used. Although this may be useful in industrial fermentations, the physical containment is generally not considered as sufficient, and additional biological containment measures are taken to reduce the possibility of survival of the genetically modified

25   microorganism in the environment. Biological containment is extremely important in cases where physical containment is difficult or even not applicable. This is, amongst others, the case in applications where genetically modified microorganisms are used as live vaccines or as vehicle for delivery of therapeutic compounds. Such applications have been described e.g. in WO 97/14806, which discloses the delivery of biologically

30   active peptides, such as cytokines, to a subject, by recombinant non-invasive or non-pathogenic bacteria. WO 96/11277 describes the delivery of therapeutic compounds to an animal – including humans – by administration of a recombinant bacterium, encoding the therapeutic protein. Steidler *et al.* (2000) describe the treatment of colitis by administration of a recombinant *Lactococcus lactis*, secreting interleukin-10. Such a

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usage of a self-containing and transform d *Lactococcus* to deliver prophylactic and/or therapeutic molecules in order to prevent and/or treat diseases.

Brief d scription of the figures

5   **Figure 1:** Map of the MG1363 *thyA* locus

Figure 2: Schematic representation of the different expression modules as present on pOThy plasmids ands genomic integrants of hIL-10. Black parts represent original *L. lactis* MG1363 genetic information, white parts represent recombinant genetic information.

10   **Figure 3:** PCR identification of Thy11 (Thy11 1.1 and Thy11 7.1 represent individually obtained, identical clones). Standard PCR reactions were performed by using aliquots of saturated cultures of the indicated strains as a source of DNA template. Panel A shows an agarose gel of the products of the indicated PCR reactions. Panel B shows the positions at which primers attach in the thyA (1), upstream (2) or downstream (3)

15   PCR's. Oligonucleotide primers used: (1): ATgACTTACgCAgATCAAgTTTTT and TTAAATTgCTAAATCAAATTTCAATTg   (2):   TCTgATTgAgTACCTTgACC   and gCAATCATAATTggTTTTATTg   (3):   CTTACATgACTATgAAAATCCg   and cTTTTTTATTATTAgggAAAgCA.

Figure 4: PCR identification of Thy11, Thy12, Thy15 and Thy16. Standard PCR

20   reactions were performed by using three days old colonies of the indicated strains as a source of DNA template.

Panel A shows the positions at which primers attach in the upstream (1), downstream (2) or thyA (3), PCR's. Oligonucleotide primers used: (1): ATgACTTACgCAgATCAAgTTTTT and TTAAATTgCTAAATCAAATTTCAATTg (2):

25   TCTgATTgAgTACCTTgACC   and   gCAATCATAATTggTTTTATTg   (3): CTTACATgACTATgAAAATCCg and cTTTTTTATTATTAgggAAAgCA

Panel B shows an agarose gel of the products of the indicated PCR reactions.

Figure 5: Southern blot analysis of the indicated strains. Chromosomal DNA was extracted and digested with the indicated restriction enzymes. Following agarose gel

30   electrophoresis the DNA was transferred to a membrane and the chromosome structure around the thyA locus was revealed by use of DIG labelled thyA or hIL-10 DNA fragments (panel A). Panel B shows a schematic overview of the predicted structure of the thyA locus in both MG1363 and Thy11.

diluted in TFM or TFM supplemented with 50μg/ml of thymidine (T50). CFU counts were determined at different time points: t=0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 20 hours. This shows that Thy12 viability is severely impaired in the absence of thymidine.

Figure 12: Intestinal passage and viability: *L. lactis* MG1363 was transformed with the
5   plasmid pLET2N which carries a chloramphenicol (Cm) resistance marker. L. lactis Thy12 was transformed with the plasmid pT1NX which carries an erythromycin (Em) resistance marker. Of both strains 10^9 bacteria were resuspended in BM9 (6 g/l Na_2HPO_4, 3 g/l KH_2PO_4, 1 g/l NH_4Cl, 0,5 g/l NaCl in 25 mM $NaHCO_3$ + 25 mM Na_2CO_3), mixed and inoculated in three mice at t=0h. Faeces were collected of the
10   time intervals -1 to 0, 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10 and 10 to overnight. All samples were resuspended in isotonic buffer and appropriate dilutions were plated on GM17 (M17 medium, Difco, St.Louis supplemented with 0,5% glucose) plates containing either Cm, Em or Em+ 50μg/ml thymidine. Colony forming units for the different plates are represented in the graph.
15

Description of the invention

It is the objective of the present invention to provide a suitable biological containment system for *Lactococcus*.

A first aspect of the invention is an isolated strain of *Lactococcus* sp. comprising a
20   defective thymidylate synthase gene. Preferably, said defective thymidylate synthase gene is inactivated by gene disruption. Even more preferably, said *Lactococcus* sp. is *Lactococcus lactis*. A special embodiment is a *Lactococcus* sp. strain, preferably *Lactococcus lactis*, more preferably a *Lactococcus lactis* MG1363 derivative, whereby the thymidylate synthase gene has been disrupted and replaced by an interleukin-10
25   expression unit. Said interleukin-10 expression unit is preferably, but not limited to, a human interleukin-10 expression unit or gene encoding for human interleukin-10.

Another aspect of the invention is the use of a strain according to the invention as host strain for transformation, whereby the transforming plasmid does not comprise an intact thymidylate synthase gene. Still another aspect of the invention is a transformed
30   strain of *Lactococcus* sp. according to the invention, comprising a plasmid that does not comprise an intact thymidylate synthase gene. Another aspect of the invention relates to a transformed strain of *Lactococcus* sp. comprising a gene or expression unit encoding a prophylactic and/or therapeutic molecule such as interleukin-10. Consequently, the present invention also relates to the usage of a transformed strain

invention further demonstrates that the transformed strains surprisingly pass the gut at the same speed as the control strains and shows that their loss of viability is indeed not different from that of the control strains. However, once said strain is secreted in the environment, e.g. in the faeces, it is not able to survive any longer.

5 The transforming plasmid can be any plasmid, as long as it cannot complement the *thyA* mutation. It may be a selfreplicating plasmid that preferably carries one or more genes of interest and one or more resistance markers, or it may be an integrative plasmid. In the latter case, the integrative plasmid itself may be used to create the mutation, by causing integration at the *thyA* site, whereby the *thyA* gene is inactivated.

10 Preferably, the active *thyA* gene is replaced by double homologous recombination by a cassette comprising the gene or genes of interest, flanked by targeting sequences that target the insertion to the *thyA* target site. It is of extreme importance that these sequences are sufficiently long and sufficiently homologous to obtain to integrate the sequence into the target site. Preferably, said targeting sequences consist of at least

15 100 contiguous nucleotides of SEQ ID N°1 at one side of the gene of interest, and at least 100 contiguous nucleotides of SEQ ID N°2 at the other side; more preferably, said targeting sequences consists of at least 500 contiguous nucleotides of SEQ ID N°1 at one side of the gene of interest, and at least 500 contiguous nucleotides of the SEQ ID N° 2 at the other side; most preferably, said targeting sequences consists of

20 SEQ ID N°1 at one side of the gene of interest and SEQ ID N°2 at the other side, or said targeting sequences consist of at least 100 nucleotides that are at least 80% identical, preferably 90% identical to a region of SEQ ID N° 1 at one side of the gene of interest, and of at least 100 nucleotides that are at least 80% identical, preferably 90% identical to a region of SEQ ID N° 2 at the other side of the gene of interest, preferably

25 said targeting sequences consist of at least 500 nucleotides that are at least 80% identical, preferably 90% identical to a region of SEQ ID N° 1 at one side of the gene of interest, and of at least 500 nucleotides that are at least 80% identical, preferably 90% identical to a region of SEQ ID N° 2 at the other side of the gene of interest, most preferably said targeting sequences consist of at least 1000 nucleotides that are at

30 least 80% identical, preferably 90% identical to a region of SEQ ID N° 1 at one side of the gene of interest, and of at least 1000 nucleotides that are at least 80% identical, preferably 90% identical to a region of SEQ ID N° 2 at the other side of the gene of interest . The percentage identity is measured with BLAST, according to Altschul *et al.* (1997). A preferred example of a s quence, homologous to SEQ ID N°1 is given in

locus as determined in the present invention are given by SEQ ID N° 19, 20, 21, 22 respectively.

The *thyA* replacement is performed by making suitable replacements in a plasmid borne version of the *thyA* target, as described below. The carrier plasmid is a

5   derivative of pORI19 (Law *et al.*, 1995) a replication defective plasmid, which only transfers the erythromycin resistance to a given strain when a first homologous recombination, at either the 5' 1000bp or at the 3'1000bp of the *thyA* target. A second homologous recombination at the 3' 1000bp or at the 5' 1000bp of the *thyA* target yields the desired strain.

10   The *thyA* gene is replaced by a synthetic gene encoding a protein which has the *L. lactis* Usp45 secretion leader (van Asseldonk *et al.*, 1990) fused to a protein of identical amino acid sequence than: (a) the mature part of human-interleukin 10 (hIL-10) or (b) the mature part of hIL-10 in which proline at position 2 had been replaced with alanine or (c) the mature part of hIL-10 in which the first two amino acids had

15   been deleted; (a), (b) and (c) are called hIL-10 analogs, the fusion products are called Usp45-hIL-10.

The *thyA* gene is replaced by an expression unit comprising the lactococcal P1 promotor (Waterfield *et al.*, 1995), the *E. coli* bacteriophageT7 expression signals: putative RNA stabilising sequence and modified gene10 ribosomal binding site (Wells

20   and Schofield, 1996).

At the 5' end the insertion is performed in such way that the ATG of thyA is fused to the P1-T7Usp45-hIL-10 expression unit.

```
5'agataggaaaatttcatgacttacgcagatcaagttttt...thyA wild type
                    gattaagtcatcttacctctt...P1-T7-usp45-hIL10
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25
```
5'agataggaaaatttcatggattaagtcatcttacctctt...thyA⁻,P1-T7-usp45-
hIL10
```

Alternatively, at the 5' end the insertion is performed in such way that the thyA ATG is not included:

30
```
5'agataggaaaatttcacttacgcagatcaagttttt...thyA wild type
                 gattaagtcatcttacctctt...P1-T7-usp45-hIL10
5'agataggaaaatttcgattaagtcatcttacctctt...thyA⁻,P1-T7-usp45-hIL10
```

integrative plasmid pT1HIL10apxa. Figure 8 (panel A and B) furth r demonstrates that all mutants produce a significant amount of h-IL 10.

Figure 9 shows the production of hIL-10 by the *L. lactis* strains LL108 carrying either pOThy11, pOThy12, or pOThy16. Quantification (by ELISA) of hIL-10 present in the
5   culture supernatant of the indicated strains. The N-terminal protein sequence of the recombinant hIL-10 was determined by Edman degradation and was shown identical to the structure as predicted for the mature, recombinant hIL-10. The protein showed full biological activity. LL108 is a *L. lactis* strain carrying a genomic integration of the repA gene, required for replication of pORI19 derived plasmids such as pOThy11,
10 pOThy12, pOThy15 or pOThy16. This strain was kindly donated by dr. Jan Kok, University of Groningen. The plasmids pOThy11, pOThy12, pOThy15 and pOThy16 carry the synthetic human IL-10 gene in different promotor configurations (see Fig. 2), flanked by approximately 1kB of genomic DNA derived from the thyA locus, upstream and downstream from thyA. These plasmids were used for the construction of the
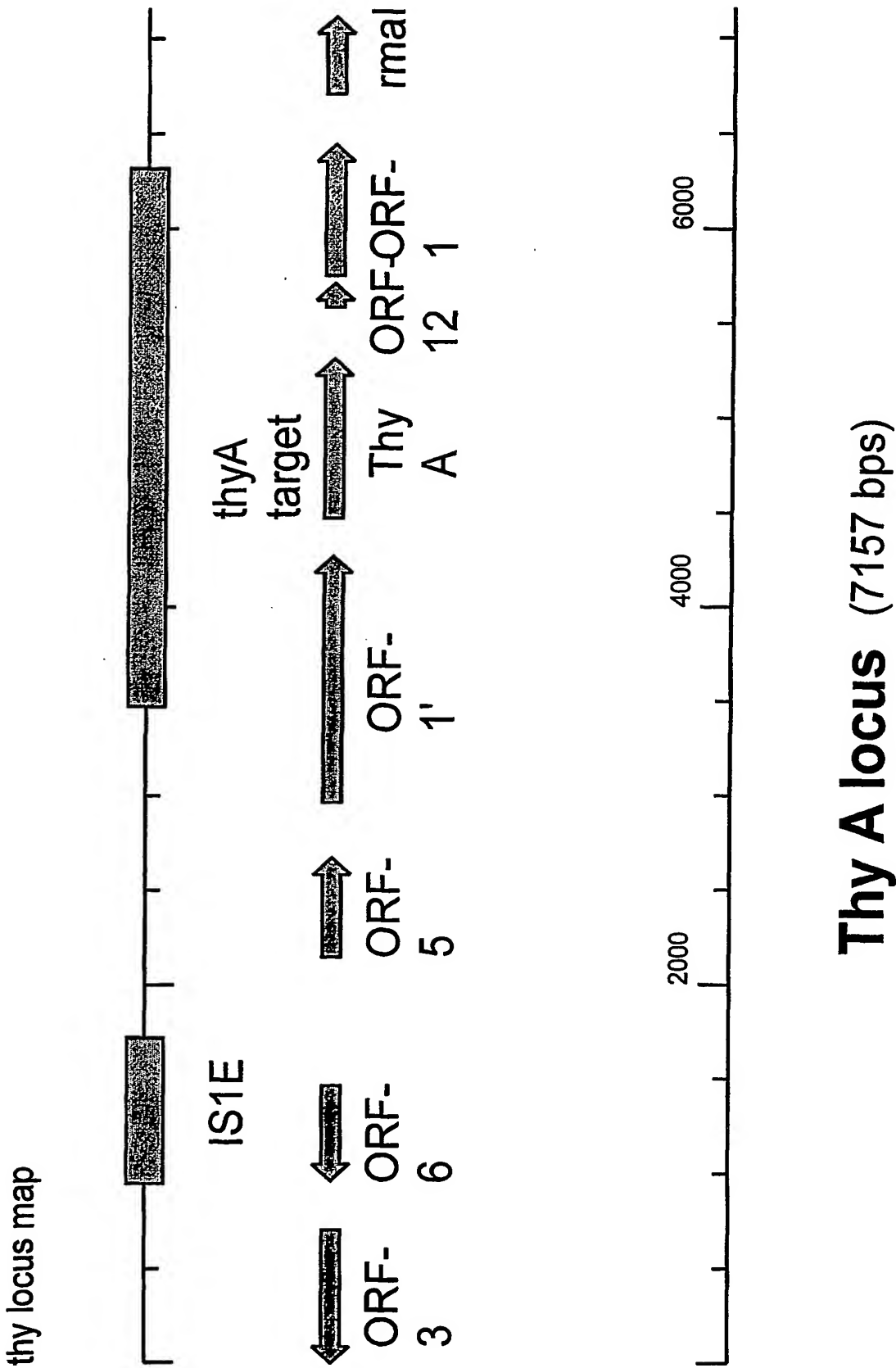15 genomic integration as described.

The effect of the thymidilate synthase deletion on the growth in thymidine less and thymidine supplemented media was tested; the results are summarized in figures 10 and 11. Absence of thymidine in the medium strongly limits the growth of the mutant,
20 and even results in a decrease of colony forming units after four hours of cultivation. Addition of thymidine to the medium results in an identical growth curve and amount of colony forming units, compared to the wild type strain, indicating that the mutant doesn't affect the growth or viability in thymidine supplemented medium. Fig. 11 clearly demonstrates that Thy12 viability is severely impaired in the absence of thymidine.
25 Fig. 12 finally shows that *L. lactis* Thy12 passes the intestine of the mice at the same speed as MG1363. Loss of viability does not appear different between Thy12 and MG1363. Thy12 appear fully dependent on thymidine for growth, indicating that no Thy12 bacteria had taken up a foreign thyA gene.
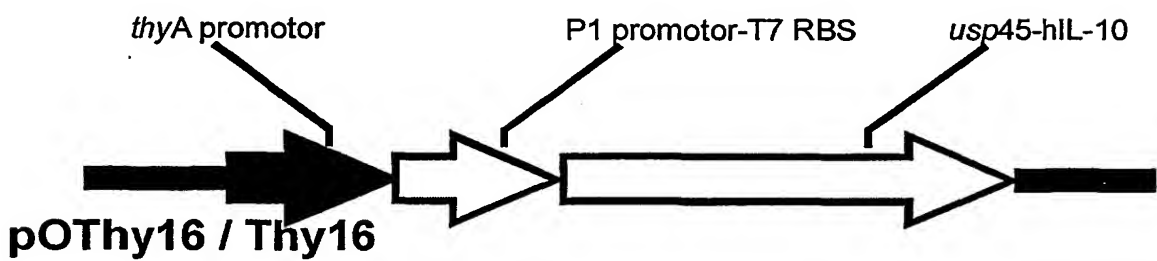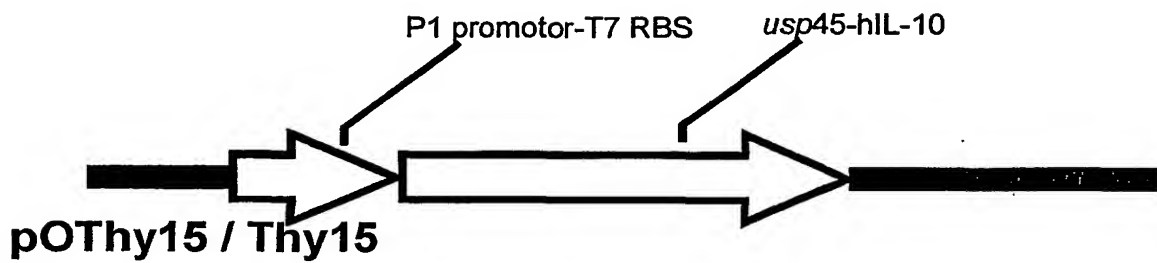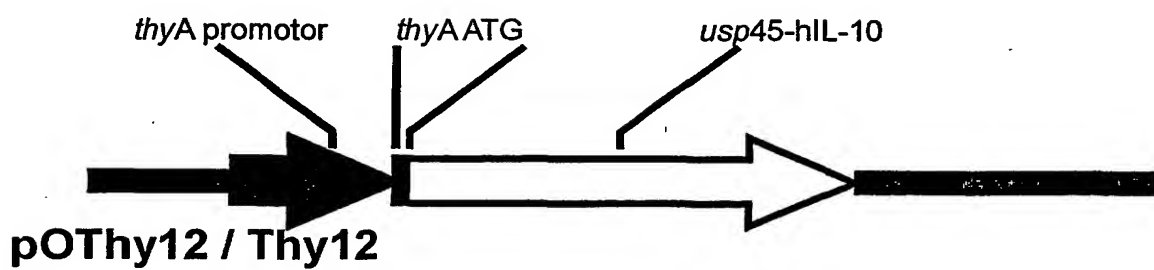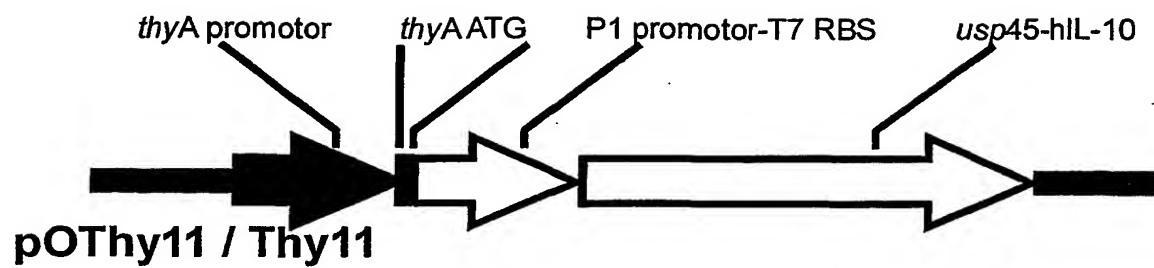
Claims

1. An isolated strain of *Lactococcus* sp. comprising a defective thymidylate synthase gene.

2. An isolated strain of *Lactococcus* sp. according to claim 1, whereby said gene is inactivated by gene disruption.

3. An isolated strain of *Lactococcus* sp. according to claim 1 or 2, whereby said *Lactococcus* sp. is *Lactococcus lactis*.

4. The use of a strain of *Lactococcus* sp. according to any of the claims 1-3 as host strain for transformation, whereby the transforming plasmid does not comprise an intact thymidylate synthase gene.

5. A transformed strain of *Lactococcus* sp. according to any of the claims 1-3, comprising a transforming plasmid that does not comprise an intact thymidylate synthase gene.

6. A transformed strain of *Lactococcus* sp. according to any of the claims 1-4 comprising a gene encoding a prophylactic and/or therapeutic molecule.

7. A transformed strain of *Lactococcus* sp. according to claim 6 wherein said prophylactic and/or therapeutic molecule is interleukin-10.

8. The use of a transformed strain of *Lactococcus* sp. according to any of the claims 5-7 for the delivery of prophylactic and/or therapeutic molecules.

9. A pharmaceutical composition comprising a transformed strain of *Lactococcus* sp. according to any of the claims 5-7.

10. The use of a transformed strain of *Lactococcus* sp. according to any of the claims 6-7 for the preparation of a medicament to treat inflammatory bowel diseases.

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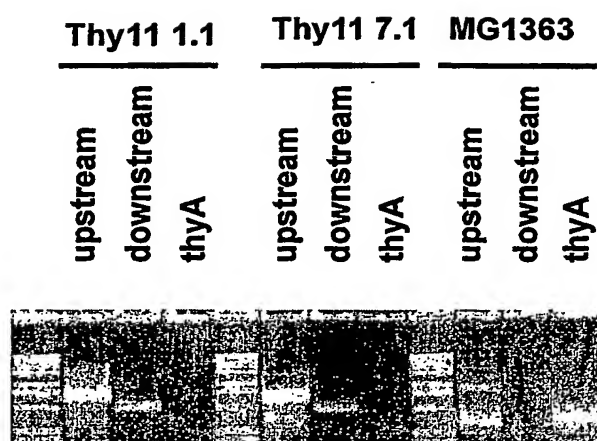
Thy A locus (7157 bps)

Figure 2:

pOThy11 / Thy11

pOThy12 / Thy12

pOThy15 / Thy15

pOThy16 / Thy16

Figure 3:

Figure 4:

Figure 5:

Figure 6:

A

B

Figure 7:

	hIL10PxA		pTREX1		Thy11 1.1		Thy11 7.1	
	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7	sample 8
concentration (ng/ml)	131,34	123,01	0	0	2,55	1,8	2,8	2,72
std (ng/ml)	0	15,27	0	0	1,05	0	0,95	0,53
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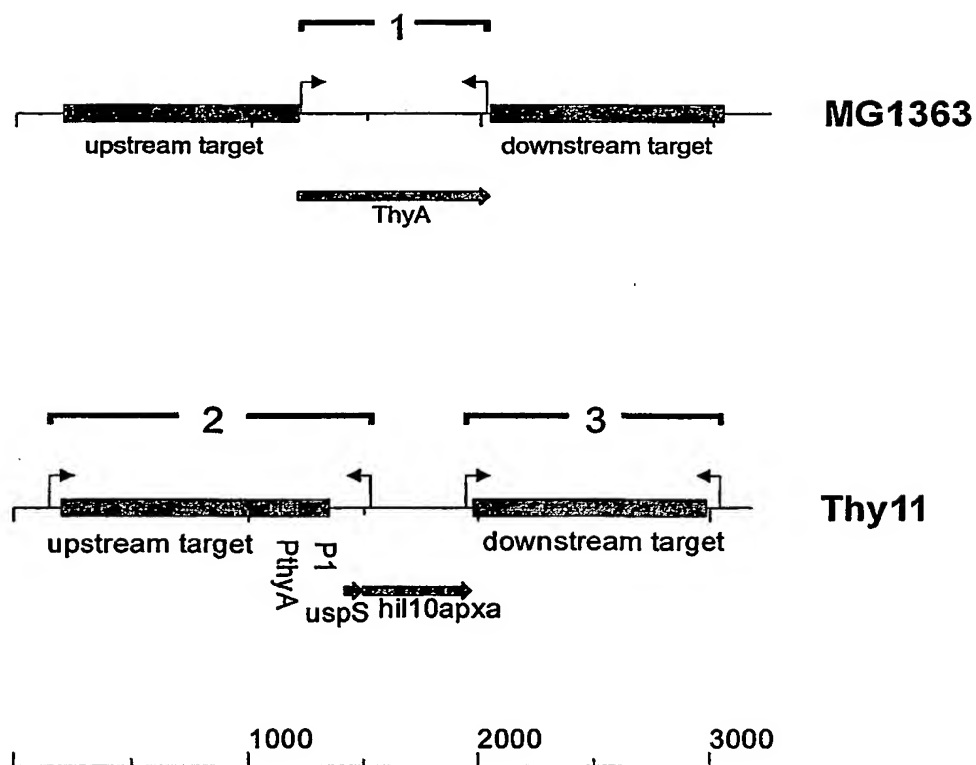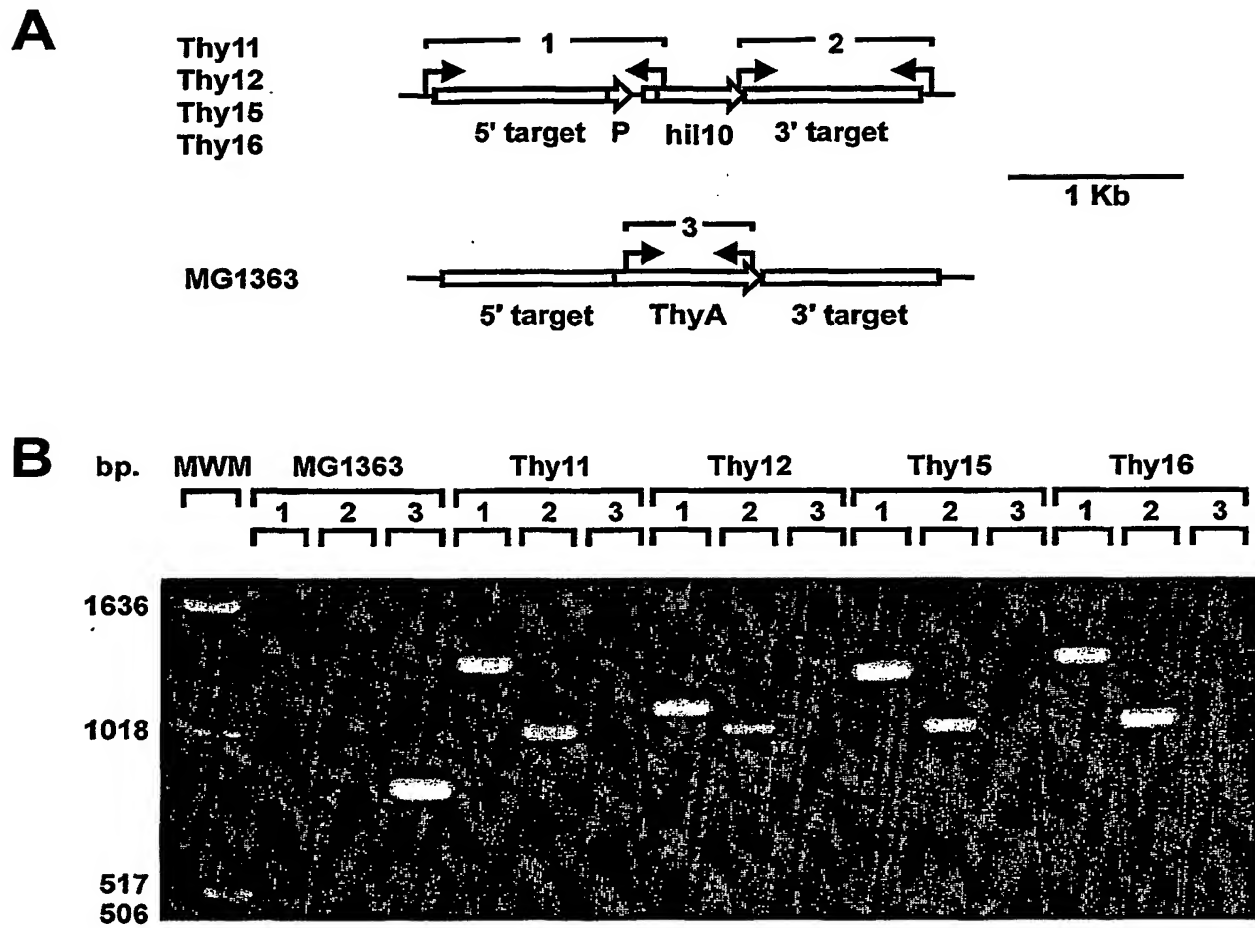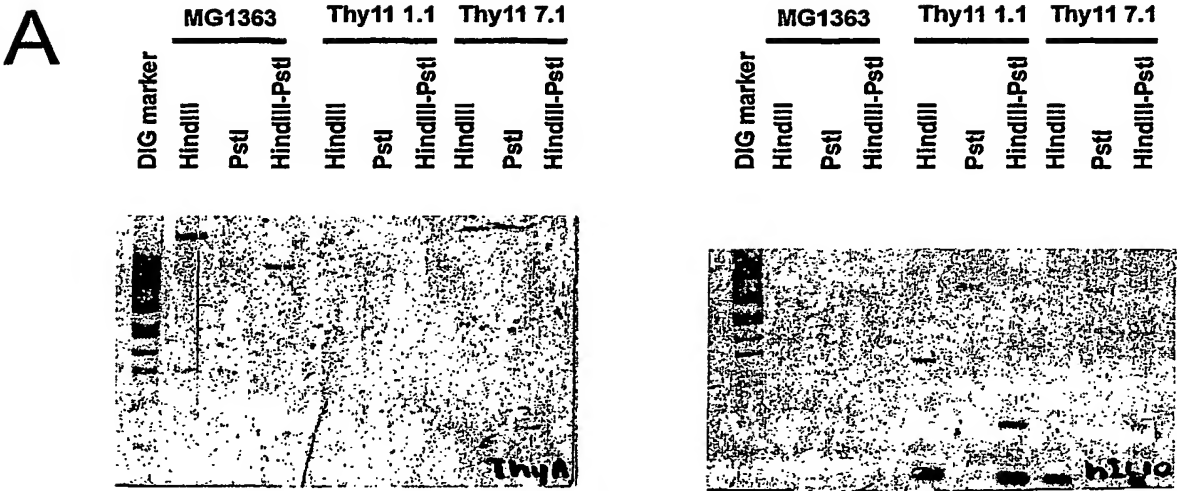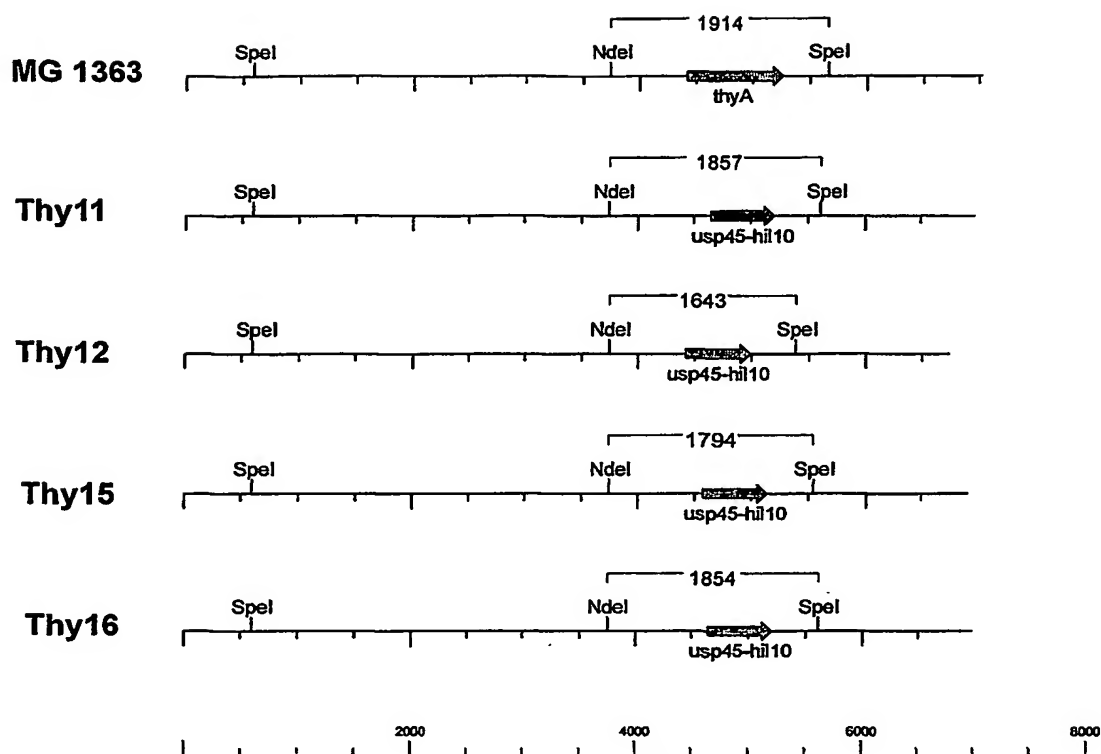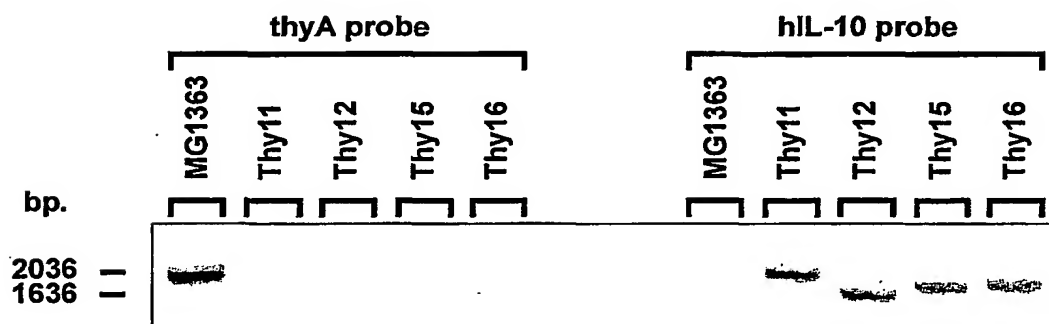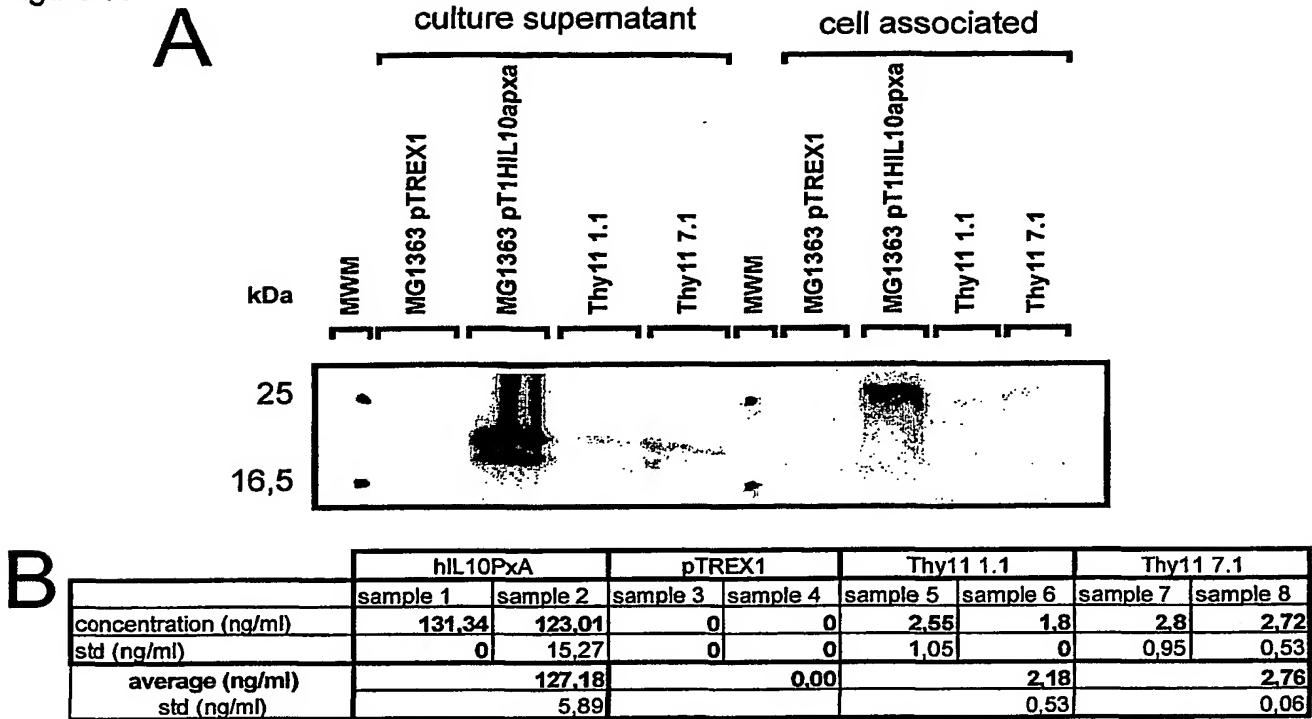
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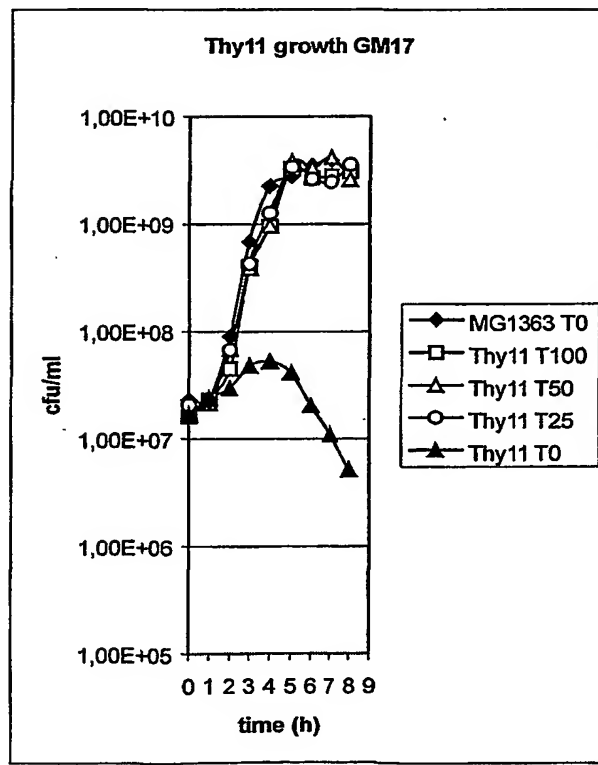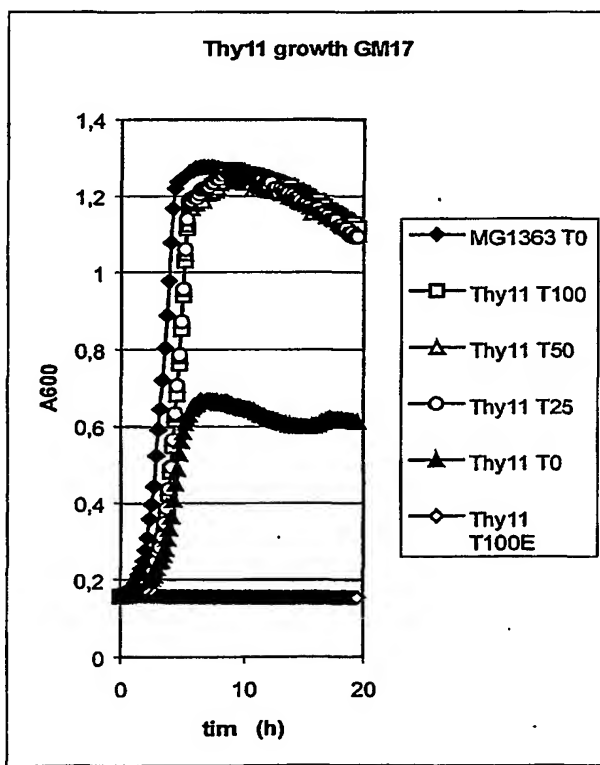
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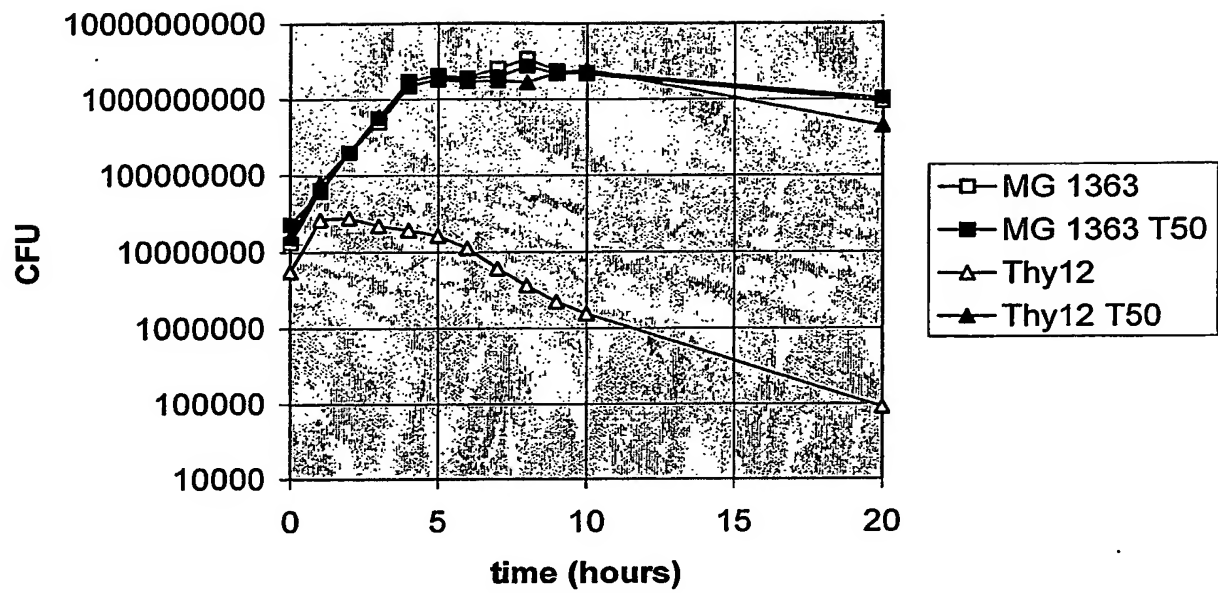
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Figure 11:

Figure 12:

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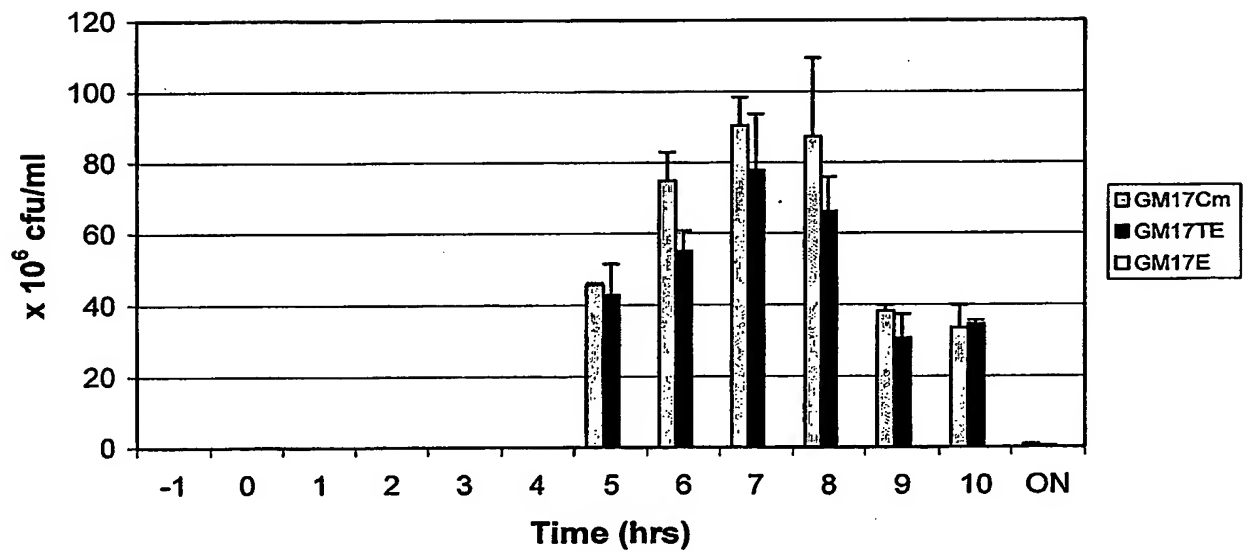
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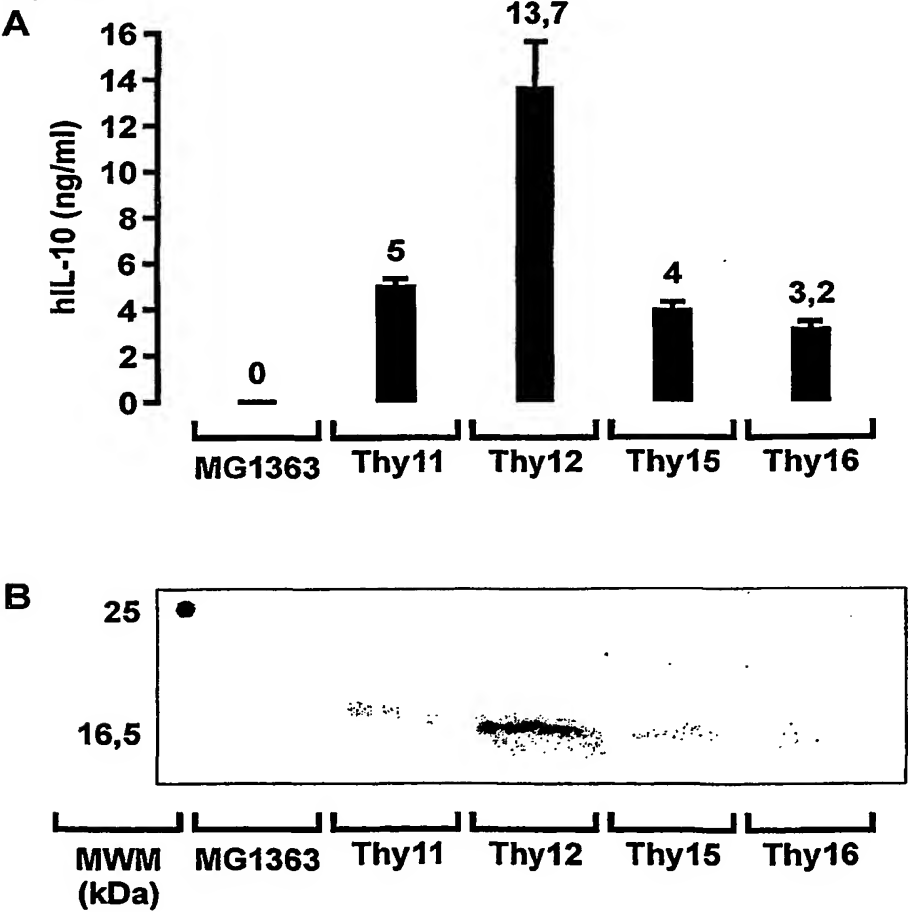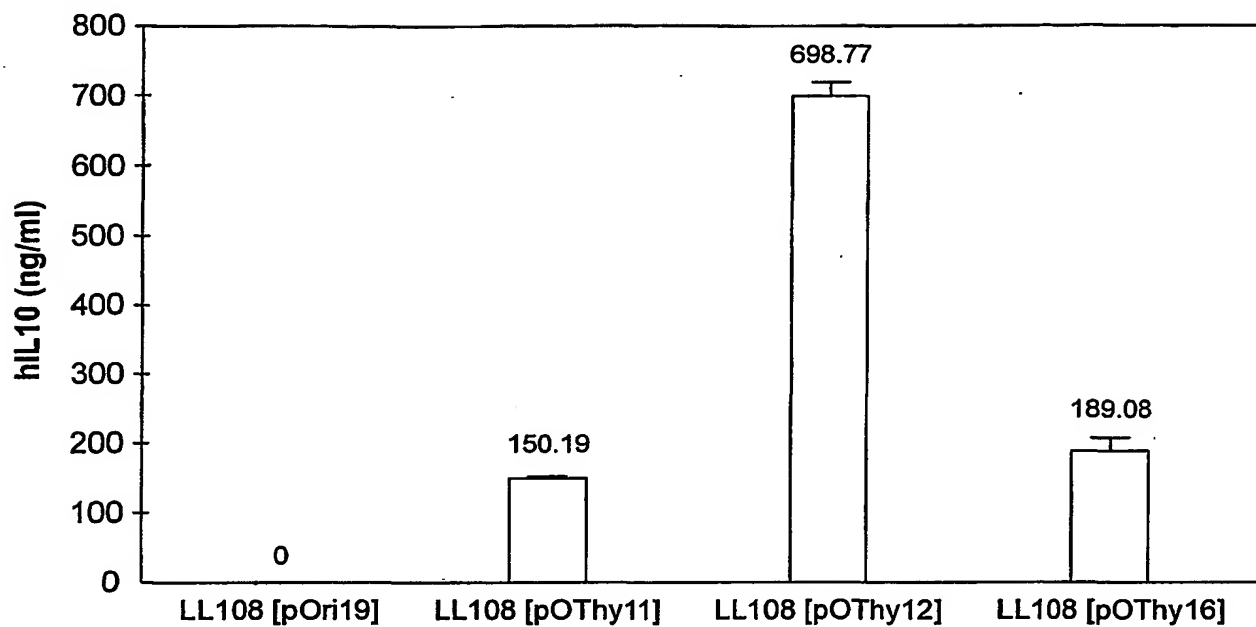tctatcctga taattctgag aggttatttt gggaaatact attgaaccat atcgaggtgt   960

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ttggacgaca ccagcacctg tgagaatggc catttcaggt ggacttccat ttttgattat       4200

ttttgcaatc tgtttagtct tgaatgttct tatttactac ccattcttta aggtggcgta       4260

taataaagct ttagaagaag aaaaagcagc tgttgaatta gagggttcag aaactgcctg       4320

atggatattt tttataaatc tggtttgaac aaattatatt gacatctctt tttctatcct       4380

gataattctg agaggttatt ttgggaaata ctattgaacc atatcgaggt gtgtggtata       4440

atgaagggaa ttaaaaaaga taggaaaatt tc atg act tac gca gat caa gtt         4493
                                     Met Thr Tyr Ala Asp Gln Val
                                     1               5

ttt aaa caa aat atc caa aat atc cta gat aat ggt gtt ttt tca gaa        4541
Phe Lys Gln Asn Ile Gln Asn Ile Leu Asp Asn Gly Val Phe Ser Glu
        10              15              20

aat gca aga cca aag tat aag gat ggt caa atg gcg aat agc aaa tat        4589
Asn Ala Arg Pro Lys Tyr Lys Asp Gly Gln Met Ala Asn Ser Lys Tyr
    25              30              35

gtc act ggt tca ttc gtt act tat gat ttg caa aag ggg gag ttt cca        4637
Val Thr Gly Ser Phe Val Thr Tyr Asp Leu Gln Lys Gly Glu Phe Pro
40              45              50              55

att acc act ttg cgt cca att cca atc aaa tct gct att aaa gaa ttg        4685
Ile Thr Thr Leu Arg Pro Ile Pro Ile Lys Ser Ala Ile Lys Glu Leu
            60              65              70

atg tgg ata tac caa gac caa aca agt gaa ctt tct gtt ctc gaa gag        4733
Met Trp Ile Tyr Gln Asp Gln Thr Ser Glu Leu Ser Val Leu Glu Glu
        75              80              85

aag tat gga gtc aaa tac tgg gga gaa tgg gga att ggt gat ggt acg        4781
Lys Tyr Gly Val Lys Tyr Trp Gly Glu Trp Gly Ile Gly Asp Gly Thr
        90              95              100

att ggg caa cgt tat ggt gca aca gtc aaa aaa tat aat atc att ggt        4829
Ile Gly Gln Arg Tyr Gly Ala Thr Val Lys Lys Tyr Asn Ile Ile Gly
105             110             115
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V085.ST25.txt

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gagatttccc ctggtagtcc acttaacatt tttgttgtac caacagaagt tgctttccct   6322

aataataaaa aagtagacga ttcaactggg caacaacgaa tttttgtgaa ttattctggt   6382

acaagccctc aaatggcgaa tagtatggca gcggtggctt tttttagagt tattccatga   6442

ttatattaaa gttagaattg aataaaatgt attattaaaa agataatatt atatcacgac   6502

aaggcgacat ctatcaactt taccactggt atggaagtga ccattattac atcaggaaac   6562

gctaaaacgg ttgttttttac acccgtaaaa taaataataa aataatgtgn aattactgac   6622

agcattttgt cagtaatttt ttttatcaaa atcacacaaa aatgttcgtt gacgaacaaa   6682

aaaaactatg ttataataat tcgtatgcga actaaaaaag aagcgattgg ccgacttta   6742

aaagtagcca gcaaccaaat gtctcgagaa tttgataatt ttgcagctca acttgatttg   6802

acaggtcagc aaatgtcaat tttagatttt cttggaaatc aaagcgaaga aggttcagga   6862

aaagaaatta gtcagacgat gattgaatta gaattaata tccgacgttc aacaacgacg   6922

gaaatttac agcgcatgga aaagcggctt ttaattaatc gaagaacaag cctgaccgat   6982

gcccgccaaa aatcagttga attaactgaa gaagggaaaa gatatttacc tgaaatcagg   7042

gcttatatcc aagcacataa taaaaaagct tggcgtaatc atggtcatag ctgtttncct   7102

ggttaggngg gccannnnn nnnnnnnnnn nnncnnnnc nnnnncnnnn cnnnc          7157
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<210> 4
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<212> PRT
<213> Lactococcus lactis

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<223> 'n' may be any base

<220>
<221> misc_feature
<222> (5)..(5)
<223> 'n' may be any base

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V085.ST25.txt

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Pro Arg Leu Val Leu Asn Val Pro Asp Gly Thr Asn Phe Phe Asp Ile
            245             250             255


Lys Pro Glu Asp Phe Glu Leu Val Asp Tyr Glu Pro Val Lys Pro Gln
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Leu Lys Phe Asp Leu Ala Ile
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<213>  Lactococcus lactis

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<222>  (4469)..(5305)
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tgctagcata tttgttgtat aatcgaacga gtccattttg aacagatcca tatagattga     180

gtgaactata aaatacatct atatcatagt tgagtttgtt cacaatcatg agaccaaatt     240

ctccagcatt tcgtgtagaa ccacgataaa gctgtttatt tagcaaaatg gcacctccga     300

cacctgtacc taaagtcatg caaataaaat tttggctttc ttgtccattc cctagccaaa     360

gttcagctag acctgcacaa ttggcatcat tttcaacata aaccggaaga tttaaatgtt     420

tttgtagttc tgtccccaat ggatagccat aaagatcagt tagagctcct gccagtaata     480

atgttccctt tttgtcagaa gttccgggaa cacttacacc aattgcagat actgaatgat     540

gagcttttaa ctgatgaata tttgtgagca agctatccat aattttttct ttttttaatg     600

gggttggaac ttgtaaatgt tgtatgatcg ttccatcact agttacaaga ccaaatttta     660

taaatgtacc accgatatca attcctattg aataatgcat cttttattac ctctttctct     720

aatttgtttt agtatagcaa aatcaaaaaa ttaattatgg tatgcattat agatatgttg     780

tataattttc acaaaaacgg agaaactat gaaacaata gaacagctca tgatagattc     840

agcagattta atgtcagatt ttattcaatt gacaatttt atattccgca aggaggattt     900

tcaacttttt tataggagtg atgaagaaga gcaagctttt tcaaggtaat gactccaact     960

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tttgagaacg acagcgactt ccgtcccagc cgtgccaggt gctgcctcag attcaggtta    1080

tgccgctcaa ttcgctgcgt atatcgcttg ctgattacgt gcagctttcc cttcaggcgg    1140
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taatgccaca aattattact gtccctgtag taaaaaacat tccaaccgaa tttccgaaat      3360

ccgcggtagt tgacagtgtg tcaaatgttg aagcatttca aacggtatac acgggtagca      3420

caggattaat tgtagcaatc ataattggtt ttattgtttc attagtctat atacaattga      3480

gcaaaagaaa tttagttatt aaattaccag ctggagttcc tccaatggtt gtagattcac      3540

taagtccagc aattatttca atggtgattt tctgtttgat gttcgggatt cgtgtgggat      3600

tctcttatac gccattccat gatattttca atttctcaac acaactaatt caagcaccgt      3660

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caattcctag tattttcaat atcagtgaac cattactttt tggtcttcca atgatgttaa      4020

atcctctttt ctttattcct tggttttcc aaccagcaat tttaggaact gtagcattgg      4080

gcttggcaaa gatattatat attacaaatc tgaatccaat gacggcactt cttccttgga      4140

cgacaccagc acctgtgaga atggccattt caggtggact tccatttttg attatttttg      4200

caatctgttt agtcttgaat gttcttattt actacccatt ctttaaggtg gcgtataata      4260

aagctttaga agaagaaaaa gcagctgttg aattagaggg ttcagaaact gcctgatgga      4320

tatttttat aaatctggtt tgaacaaatt atattgacat ctctttttct atcctgataa      4380

ttctgagagg ttattttggg aaatactatt gaaccatatc gaggtggtgt ggtataatga      4440

agggaattaa aaaagatagg aaaatttc atg act tac gca gat caa gtt ttt       4492
                                  Met Thr Tyr Ala Asp Gln Val Phe
                                   1               5

aaa caa aat atc caa aat atc cta gat aat ggt gtt ttt tca gaa aat       4540
Lys Gln Asn Ile Gln Asn Ile Leu Asp Asn Gly Val Phe Ser Glu Asn
     10              15                  20

gca aga cca aag tat aag gat ggt caa atg gcg aat agc aaa tat gtc       4588
Ala Arg Pro Lys Tyr Lys Asp Gly Gln Met Ala Asn Ser Lys Tyr Val
25                  30                  35                  40

act ggt tca ttc gtt act tat gat ttg caa aag ggg gag ttt cca att       4636
Thr Gly Ser Phe Val Thr Tyr Asp Leu Gln Lys Gly Glu Phe Pro Ile
                 45                  50                  55

acc act ttg cgt cca att cca atc aaa tct gct att aaa gaa ttg atg       4684
Thr Thr Leu Arg Pro Ile Pro Ile Lys Ser Ala Ile Lys Glu Leu Met
                 60                  65                  70

tgg ata tac caa gac caa aca agt gaa ctt tct gtt ctc gaa gag aag       4732
Trp Ile Tyr Gln Asp Gln Thr Ser Glu Leu Ser Val Leu Glu Glu Lys
         75                  80                  85

tat gga gtc aaa tac tgg gga gaa tgg gga att ggt gat ggt acg att       4780
Tyr Gly Val Lys Tyr Trp Gly Glu Trp Gly Ile Gly Asp Gly Thr Ile
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                                        V085.ST25.txt
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ctcaaatggc  gaatagtatg  gcagcggtgg  cttttttttag  agttattcca  tgattatatt    6445

aaagttagaa  ttgaataaaa  tgtattatta  aaaagataat  attatatcac  gacaaggcga    6505

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cggttgtttt  tacacccgta  aaataaataa  taaaataatg  tgaaattact  gacagcattt    6625

tgtcagtaat  ttttttttatc  aaaatcacac  aaaaatgttc  gttgacgaac  aaaaaaaact    6685

atgttataat  aattcgtatg  cgaactaaaa  aagaagcgat  tggccgactt  ttaaaagtag    6745

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tacagcgcat  ggaaaagcgg  cttttaatta  atcgaagaac  aagcctgacc  gatgcccgcc    6985

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<212> PRT
<213> Lactococcus lactis

<400> 6

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Asp Asn Gly Val Phe Ser Glu Asn Ala Arg Pro Lys Tyr Lys Asp Gly
            20                  25                  30


Gln Met Ala Asn Ser Lys Tyr Val Thr Gly Ser Phe Val Thr Tyr Asp
        35                  40                  45


Leu Gln Lys Gly Glu Phe Pro Ile Thr Thr Leu Arg Pro Ile Pro Ile
    50                  55                  60


Lys Ser Ala Ile Lys Glu Leu Met Trp Ile Tyr Gln Asp Gln Thr Ser
65                  70                  75                  80


Glu Leu Ser Val Leu Glu Glu Lys Tyr Gly Val Lys Tyr Trp Gly Glu
                85                  90                  95
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V085.ST25.txt

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taaaattagg tgcaattcct agtattttca atatcagtga accattactt tttggtcttc     540

caatgatgtt aaatcctctt ttctttattc ctttggtttt ccaaccagca attttaggaa     600

ctgtagcatt gggcttggca aagatattat atattacaaa tctgaatcca atgacggcac     660

ttcttccttg gacgacacca gcacctgtga gaatggccat ttcaggtgga cttccatttt     720

tgattatttt tgcaatctgt ttagtcttga atgttcttat ttactaccca ttctttaagg     780

tggcgtataa taaagcttta gaagaagaaa aagcagctgt tgaattagag ggttcagaaa     840

ctgcctgatg gatatttttt ataaatctgg tttgaacaaa ttatattgac atctcttttt     900

ctatcctgat aattctgaga ggttattttg ggaaatacta ttgaaccata tcgaggtggt     960

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V085.ST25.txt

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agatccatat agattgagtg aactataaaa tacatctata tcatagttga gtttgttcac   180

aatcatgaga ccaaattctc cagcatttcg tgtagaacca cgataaagct gtttatttag   240

caaaatggca cctccgacac ctgtacctaa agtcatgcaa ataaaatttt ggctttcttg   300

tccattccct agccaaagtt cagctagacc tgcacaattg gcatcatttt caacataaac   360

cggaagattt aaatgttttt gtagttctgt ccccaatgga tagccataaa gatcagttag   420

agctcctgcc agtaataatg ttcccttttt gtcagaagtt ccgggaacac ttacaccaat   480

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gcattataga tatgttgtat aattttcaca aaaacggaga aaactatgaa aacaatagaa   780

cagctcatga tagattcagc agatttaatg tcagatttta ttcaattgac aatttttata   840

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catgcagctc accgatttt gagaacgaca gcgacttccg tcccagccgt gccaggtgct   1020

gcctcagatt caggttatgc cgctcaattc gctgcgtata tcgcttgctg attacgtgca   1080

V085.ST25.txt

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cgcaagtttg ttaatgctaa tgccacaaat tattactgtc cctgtagtaa aaaacattcc      3300

aaccgaattt ccgaaatccg cggtagttga cagtgtgtca aatgttgaag catttcaaac      3360

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V085.ST25.txt
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agatccatat agattgagtg aactataaaa tacatctata tcatagttga gtttgttcac     180

aatcatgaga ccaaattctc cagcatttcg tgtagaacca cgataaagct gtttatttag     240

caaaatggca cctccgacac ctgtacctaa agtcatgcaa ataaaatttt ggctttcttg     300

tccattccct agccaaagtt cagctagacc tgcacaattg gcatcatttt caacataaac     360

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